

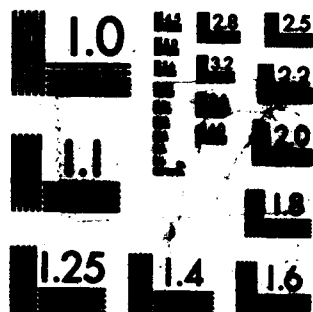
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EFFECTIVENESS AND MECHANISMS OF ANTAGONISM OF TOXIC
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CENTER JACKSON DEPT OF PHARMACOLOGY A. S. HUME
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**EFFECTIVENESS AND MECHANISM OF ANTAGONISM OF
TOXIC EFFECTS OF CYANIDE BY α -KETO ACIDS**

FINAL REPORT

(September 15, 1985-September 14, 1986)

Arthur S. Hume, Ph.D.

December 31, 1986

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**Department of Pharmacology and Toxicology
The University of Mississippi Medical Center
2500 North State Street
Jackson, Mississippi 39216-4505**

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| <p>The purpose of this project has been to investigate the development of an antidotal and/or prophylactic agent to antagonize the toxic effects of cyanide (CN). The work centered around carbonyl containing chemicals - alpha-keto acids. Alpha-ketobutyric (alpha-KB), glutamic, alpha-ketoglutaric (alpha-KG), B-ketoglutaric (B-KG), dehydroascorbic and pyruvic (PHY) acids were studied. Alpha-Ketoacids were shown to bind cyanide in vitro. Evidence of this binding is the decreased concentration of alpha-keto acids and the decreased quantities of cyanide released from mixtures of potassium cyanide and alpha-keto acids. Adult mice, rats and dogs (laboratory raised) have been dosed with alpha-keto acids both prophylactically and antidotally by intraperitoneal administration. Alpha-KG showed the least toxicity (up to 4.0 grams/kg) although other keto acids were slightly more effective as antidotes. Studies into the mechanism have been done, alpha-KG does prevent the inhibition of cytochrome oxidase by cyanide and has no effect on the transulfurase, rhodanase. Alpha-KG does not enhance the formation of methemoglobin or thiocyanate.</p> | | | |
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19. Abstract (con't)

In studies involving the dogs, nine cardiovascular and thirteen blood gas parameters were measured and evaluated for the antagonistic effects of alpha-KG on cyanide toxicity. In the dog, alpha-KG reverses some of the toxic effects of cyanide on the heart (particularly arrhythmias). Also, the administration of alpha-KG prevents circulatory collapse and blood pressure was also maintained during cyanide infusion to these animals. Metabolic acidosis, a toxic effect of cyanide, did not appear until the animals were near death. Lethal blood levels of cyanide in alpha-KG treated animals approached levels of 5-7 mcg% cyanide, which is 5-7 times the expected lethal levels. From these studies, alpha-KG is effective in antagonizing administered dose of CN of five times the lethal dose before the toxic effects are irreversible.

FOREWORD

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals", Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. [NIH] 86-23, Revised 1985).

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I. Toxicity Studies of Keto Acid Compounds

Groups of five male ICR mice (20-24 g) were used. Solutions of the acids; α -ketoglutaric (α -KG), α -ketobutyric (α -KB), pyruvic (PYR), β -ketoglutaric (β -KG) and dehydroascorbic (DEH) were used. The solutions were injected i.p. at a minimum volume of 0.2 ml.

TABLE 1.

Lethality of Keto Acids

| Acid | Dose | | |
|--------------|----------|----------|----------|
| | 1.0 g/kg | 2.0 g/kg | 4.0 g/kg |
| α -KG | 0/5 | 0/5 | 0/5 |
| α -KB | 0/5 | 0/5 | |
| PYR | 0/5 | 0/5 | |
| β -KG | 0/5 | 0/5 | |
| DEH | 2/5 | 3/5 | |

Results are expressed as dead animals over total animals used.

These studies show that only DEH acid produced deaths in mice in the doses used. α -KB, and PYR acids were not lethal to any mice at the 1.0 and 2.0 g/kg doses used. Previous research with α -KG acid had shown that a dose of 2.0 g/kg could be used in studies of antagonism of CN in mice. α -KG could be administered in a saturated solution at a dose level of 4.0 g/kg with only slight lethargy and tremors noted as the result.

II. Studies on the Binding of Cyanide by α -Keto Acids

A. Studies by ultraviolet spectrophotometry

In the first experiments, ultraviolet spectra of solutions of 0.05 M concentrations of α -KG, α -KB, and β -KG were obtained. A spectrum of potassium cyanide (KCN) was also obtained. Increasing concentrations of CN were added to solutions of the keto acids and the alterations in UV spectra noted (Figures 1-4).

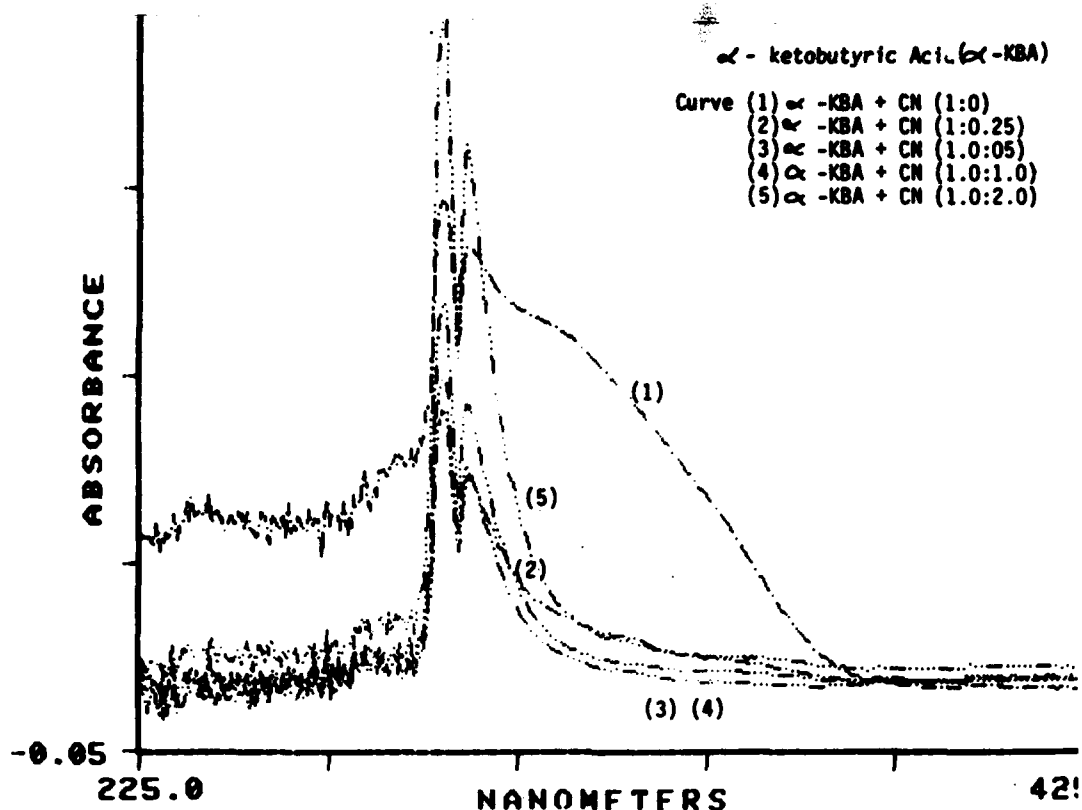


FIGURE 1.

Ultraviolet spectra of β -ketoglutaric acid and increasing concentration of potassium cyanide.

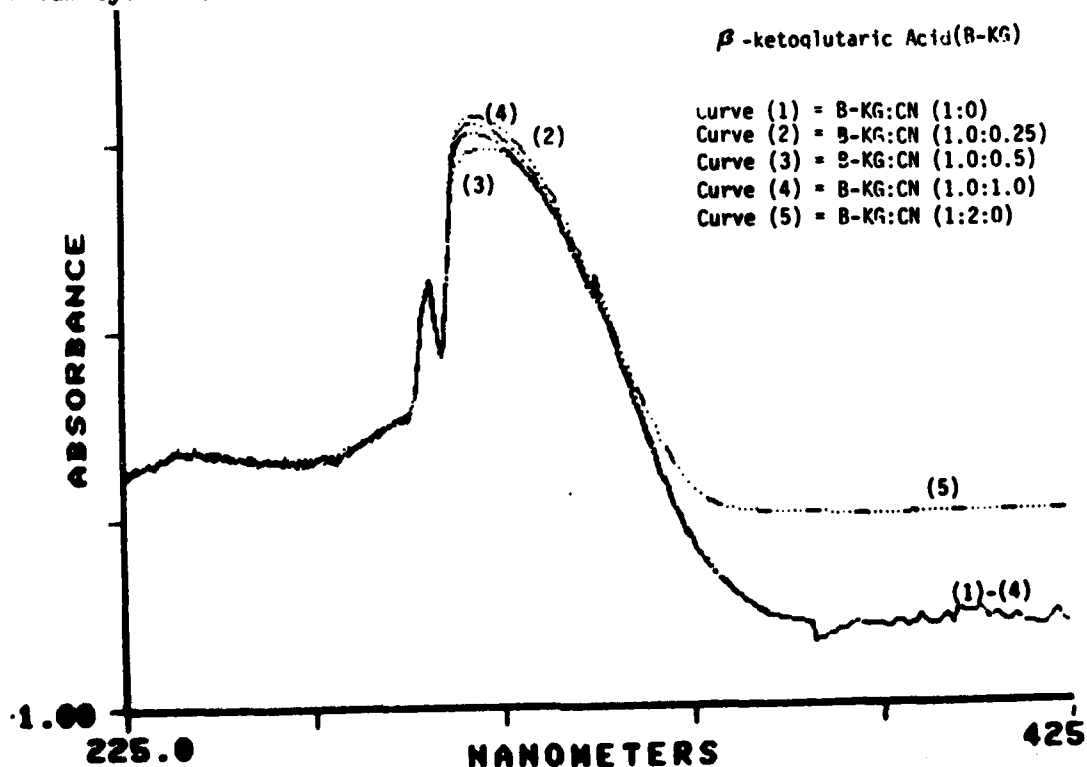


FIGURE 2.

Ultraviolet spectra of α -ketobutyric acid and increasing concentrations of potassium cyanide.

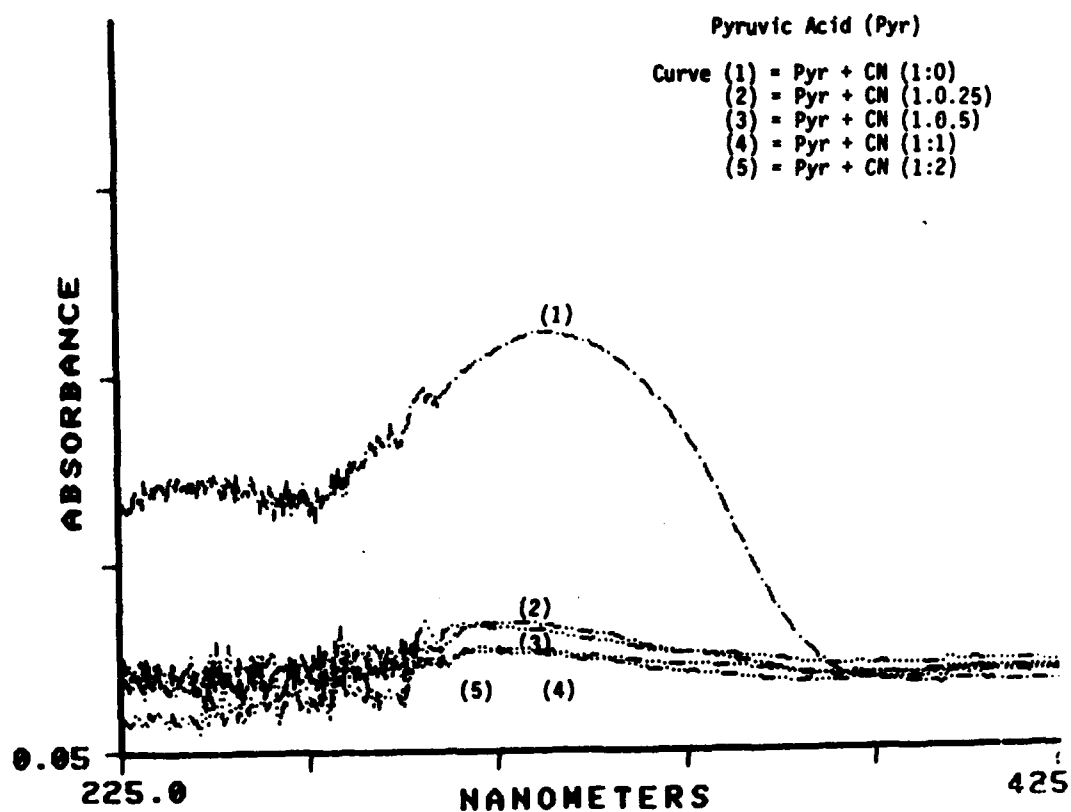


FIGURE 3.

Ultraviolet spectra of pyruvic acid and increasing concentrations of potassium cyanide.

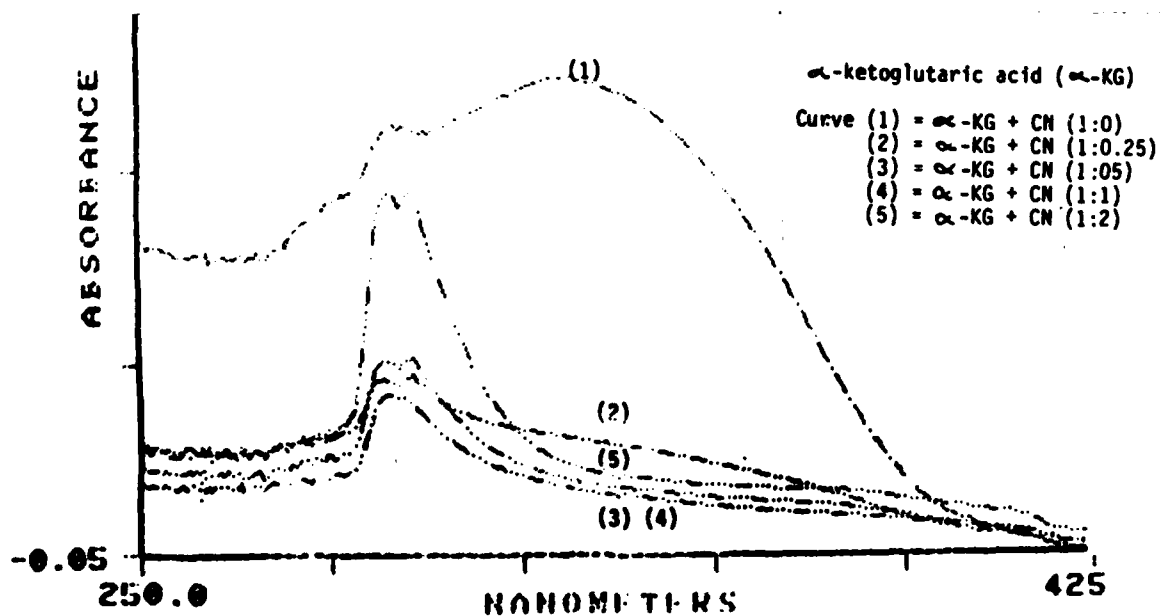


FIGURE 4.

Ultraviolet spectra of α -ketoglutaric acid and increasing concentrations of potassium cyanide.

From these spectra it can be seen that the keto acid chromophores of α -KG and α -KB are diminished greatly when KCN is added. A new chromophore is observed at 288 nm. This chromophore is proposed to be the cyanohydrin of each alpha keto acid.

Thus, it can be concluded that α -KG, PYR and α -KB bind with cyanide spontaneously and to a very high extent. In fact, the α -keto acid disappears, indicating a very desirable degree of binding cyanide for antidotal purposes. It is also concluded that β -KG does not bind to CN since the spectrum of β -KG is essentially unchanged by the increasing amounts of cyanide present.

B. Studies by High Performance Liquid Chromatography

Evidence of this binding was also obtained by a method using high performance liquid chromatography (Waters, Model 440). The column was an Interaction^R ORH-801 Organic Acid. The detector was electrochemical equipped with a glassy carbon electrode. The mobile phase was 0.03 N H_2SO_4 . The α -keto acids were injected into this system in the absence of CN. The peak area of each α -keto acid was measured. Then various molar ratios of α -keto acid:CN were injected into the HPLC system and the peak area of the α -keto acid measured. The peak areas of the α -keto acid without CN and with CN were compared. Any reduction in the peak area of the α -keto acid in the presence of CN was assumed to be due to binding of the α -keto acid and CN.

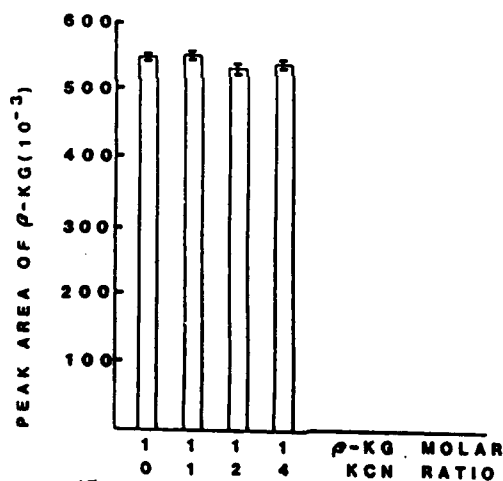


FIGURE 5.

In this figure the y-axis represents the peak area of β -ketoglutaric acid. The x-axis illustrates the combination of β -ketoglutaric acid and cyanide analyzed.

It can be observed from Figure 5 that there is no reduction in the peak area of β -KG with increasing concentrations of KCN. Thus, it is concluded that no binding reaction between β -KG and CN has occurred.

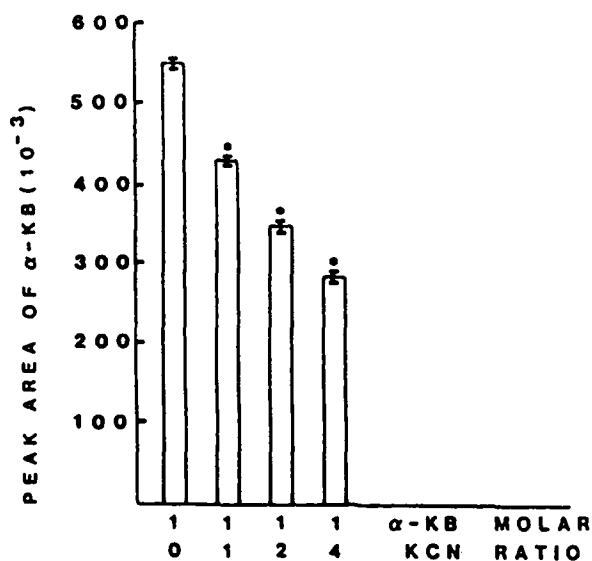


FIGURE 6.

In this figure the y-axis represents the peak area of α -ketobutyric acid. The x-axis illustrates the combination of molar ratios of α -ketobutyric acid and cyanide analyzed.

From Figure 6 it can be concluded that the concentration of KCN does affect the peak area of the α -KB response. This indicates that binding of α -KB and cyanide does occur. At a concentration ratio of 1:4 the peak area is reduced about 50%.

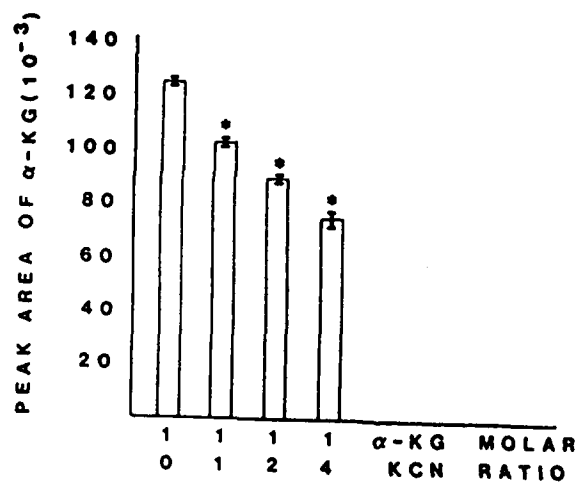


FIGURE 7.

In this figure the y-axis represents the peak area of α -ketoglutaric acid. The x-axis illustrates the combination of molar ratios of α -ketoglutaric acid and cyanide analyzed.

From Figure 7, the presence of potassium cyanide affected the peak area of α -KG. Thus, α -KG concentration is reduced. At a ratio of 1:4, the α -KG concentration is reduced approximately 40%, indicating that CN does bind α -KG.

III. Effects of α -Ketoglutaric Acid (α -KG) on Distribution of Cyanide (CN) in Mice

If (α -KG) does, indeed, bind CN in vitro, then a possible mechanism of antagonism of CN intoxication could be the binding of CN by α -KG in the blood. This binding of CN should result in a retention of CN in the circulating blood and a reduction in distribution of CN to tissues.

In order to test this hypothesis, experiments using radioactive potassium cyanide were designed to determine the effects of binding CN by α -KG upon the distribution of CN. Radioactive potassium cyanide (^{14}C) was obtained from Amersham. One group of animals served as controls and received only potassium cyanide. The test group of animals received α -KG (1.0 gm/kg). These animals were injected with (α -KG) 10 minutes prior to cyanide challenge. Both groups cyanide challenge, animals were decapitated at 2.5, 5.5, 8.5 and 11.5 minutes after the dose of cyanide. After homogenization and preparation of samples, the radioactivity was determined by counting carbon 14 using a scintillation counter (Beckman LS1800). The results of these experiments are shown in Figures 8, 9, 10 and 11.

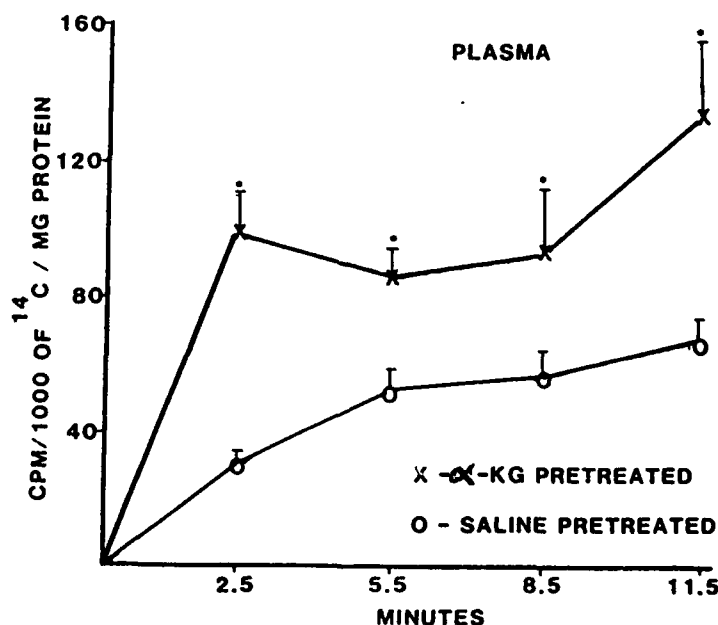


FIGURE 8.

The effect of α -ketoglutaric acid pretreatment upon the distribution of cyanide into the plasma over time.

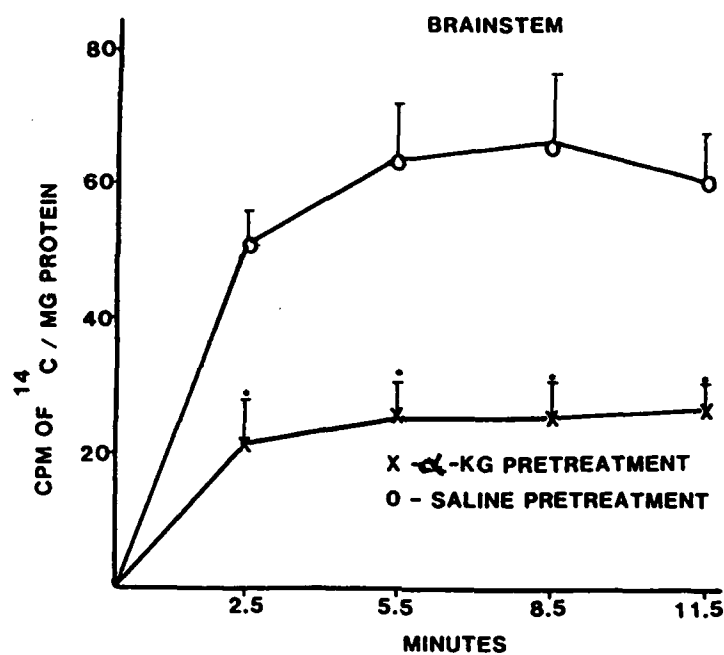


FIGURE 9.

The effect of α -ketoglutaric acid pretreatment upon the distribution of cyanide into the brainstem over time.

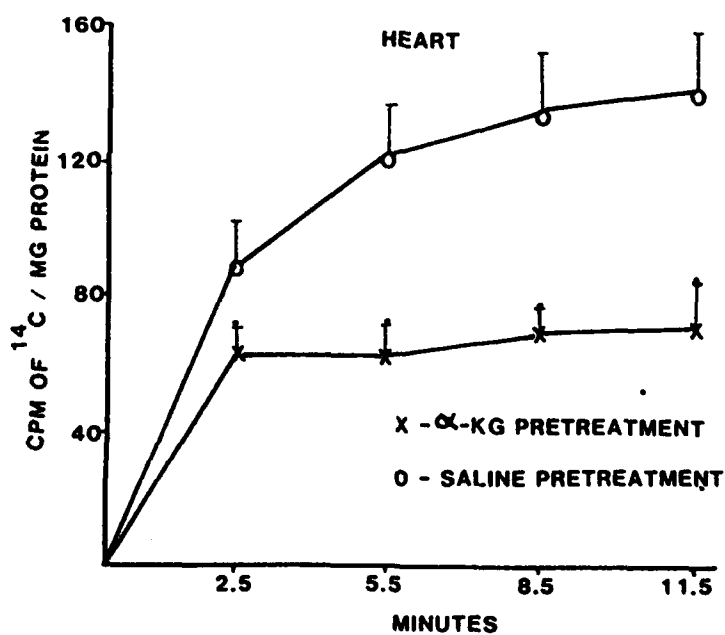


FIGURE 10.

The effect of α -ketoglutaric acid pretreatment upon the distribution of cyanide to the heart over time.

The figures present data that α -KG does inhibit the movement of cyanide out of the plasma into the tissues. From Figure 8, the plasma levels of cyanide of treated animals are two to three times that of untreated animals. Whereas, in brainstem and heart tissue, the levels of cyanide are one-third to one-half that of the animals in the α -KG treated group which had received only cyanide. By binding CN in the plasma, α -KG would inhibit the movement of CN into the tissues and exerting its toxic effects on the enzyme systems. In this manner, α -KG would function as an antidote for cyanide poisoning.

IV. The Effect of Keto Acids on Lethality Produced by Cyanide

The objective of these experiments was to determine the ability of α -keto acids to prevent cyanide-induced lethality. Male ICR mice (20-24 g) were injected with the respective α -keto acids i.p. 15 minutes prior to the i.p. injection of potassium cyanide. A lethality curve for potassium cyanide was obtained by injecting mice with increasing doses of potassium cyanide i.p. The LD_{50} values were calculated according to the method of Litchfield and Wilcoxon (1947). The results are shown in Figure 11.

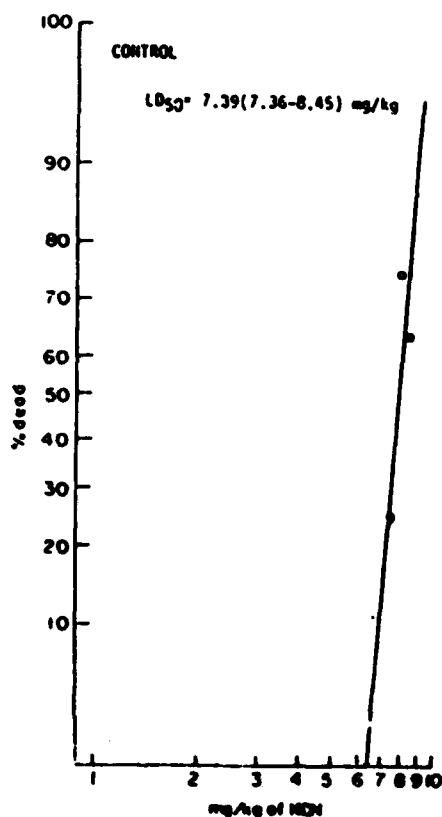


FIGURE 11.

A plot of dosage of KCN versus % death in animals.

-Dehydroascorbic, glutamic, α -ketovaleric, α -ketoglutaric, α -ketobutyric, α -ketoglutaric and pyruvic acids were obtained from Sigma Chemical Company, St. Louis, MO. The α -keto acids were administered (equimolar doses, 0.0238 M) 15 minutes prior to the injection of potassium cyanide (an LD_{50} dose, 9.5 mg/kg. i.p.). The animals were observed for 24 hours for toxic and lethal effects.

TABLE 2.

Protection against Lethal Effects of Cyanide by Keto Acids

| Lethality Results Ratio | |
|-------------------------|------|
| Saline | 9/10 |
| β -KGA | 9/10 |
| α -KBA | 1/10 |
| α -KGA | 2/10 |
| PYR | 5/10 |
| GLU | 8/10 |
| DEH | 3/10 |

*Lethality results are expressed as number of dead animals over total number of animals used.

α -KG and α -KB were observed to be the most effective protectants against the lethality produced by cyanide. Only two, and one animals, respectively, died when injected with an LD₅₀ of potassium cyanide after treatment with these α -keto acids. PYR was effective in protecting five of the animals while in the dehydroascorbic acid-treated animals, three of the ten animals survived.

Glutamic acid was nearly ineffective and β -ketoglutaric acid was totally ineffective in increasing the survivability of the cyanide-treated animals.

TABLE 3.

LD₅₀ Values of Potassium Cyanide in Pretreated Animals

| Pretreatment | LD ₅₀ Values of Potassium Cyanide (mg/kg, i.p.) [95% confidence intervals] |
|--------------|--|
| Saline | 7.39 [6.50-8.94] |
| PYR | 9.48 [8.42-10.62] |
| β -KG | 7.69 [7.31-8.24] |
| α -KB | 13.04 [11.77-15.32] |
| α -KG | 23.94 [21.36-27.90] |

Table 3 shows the LD₅₀ values of potassium cyanide in the keto acid-pretreated animals. The β -KG pretreatment did not elevate the LD₅₀ value of KCN. However, α -KB pretreatment significantly elevated the LD₅₀ value of KCN to 13.04 mg/kg. Also, α -KG pretreatment significantly elevated the LD₅₀ value of KCN to 23.94 mg/kg.

V. The Effects of Keto Acids on Cytochrome Oxidase Activity

Since the inhibition of the enzyme, cytochrome oxidase, is a toxic effect of cyanide, an antagonist of cyanide should prevent this inhibition. To evaluate this hypothesis, cytochrome oxidase activity was assayed utilizing the spectrophotometric method of Cooperstein and Lazarow (1950). Naive mice brains were used in the study. Brain homogenates were grouped as control, CN-treated and keto acid plus CN treated. The enzymic activity was monitored as a decrease in the absorbance of this mixture at 550 nm with a Cary 17 spectrophotometer. Protein concentrations were determined by the method of Bradford (1976). In Figure 12 is shown that various concentrations of α -KG prevent cyanide-induced inhibition of brain cytochrome oxidase. Concentration of 0.05 and 0.06 M α -KG prevented 100% of the CN-induced inhibition of cytochrome oxidase activity. The activity of brain cytochrome oxidase could be inhibited 100% by cyanide at a concentration of 10^{-5} M.

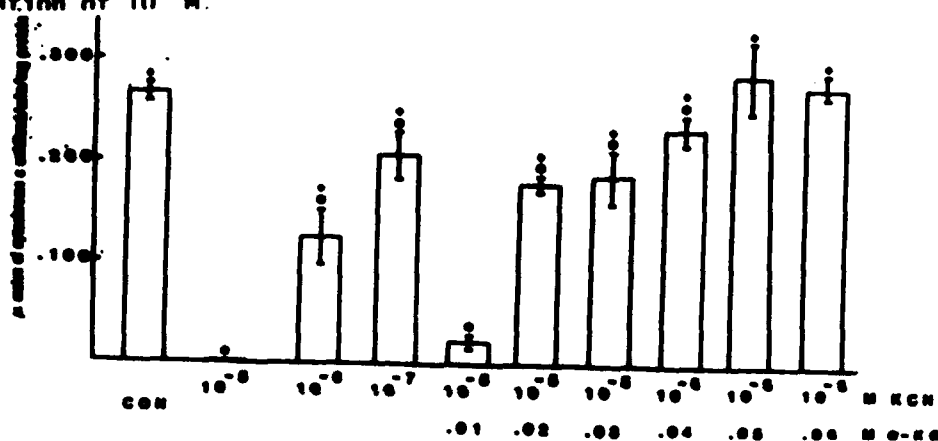


FIGURE 12.

Prevention of cyanide (-KCN) inhibition of cytochrome oxidase by α -ketoglutaric acid (α -KG).

Thus, α -KG is shown to be very effective in preventing CN inhibition of cytochrome oxidase in these in vitro studies.

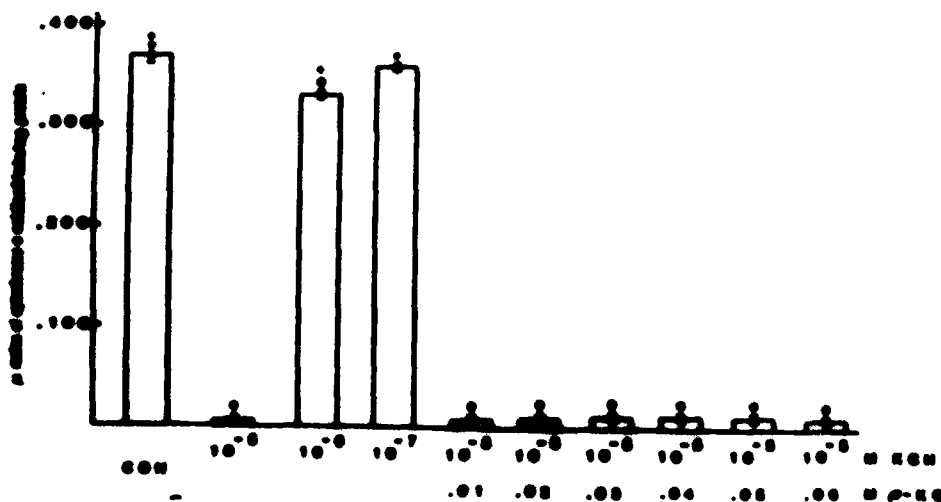


FIGURE 13.

Prevention of cyanide (CN) inhibition of cytochrome oxidase by α -ketoglutaric acid (α -KG).

In Figure 13 none of the concentrations of β -KG prevented cyanide-induced inhibition of brain cytochrome oxidase activity.

Another α -keto acid, (α -KB) was shown to prevent the cyanide inhibition of cytochrome oxidase.

Concentrations of 0.05 and 0.06 M α -KB prevented 75% of the cyanide-induced inhibition (Figure 14).

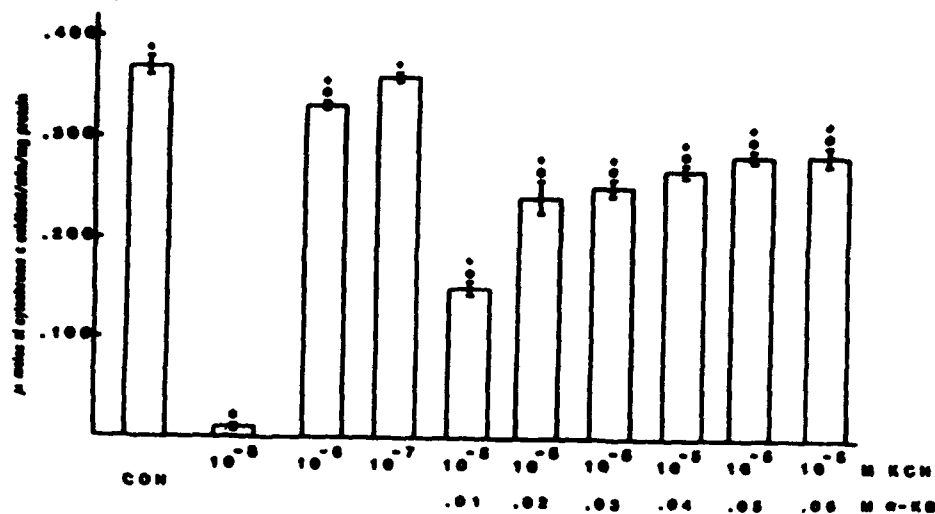


FIGURE 14.

Prevention of cyanide inhibition of cytochrome oxidase by α -ketobutyric acid.

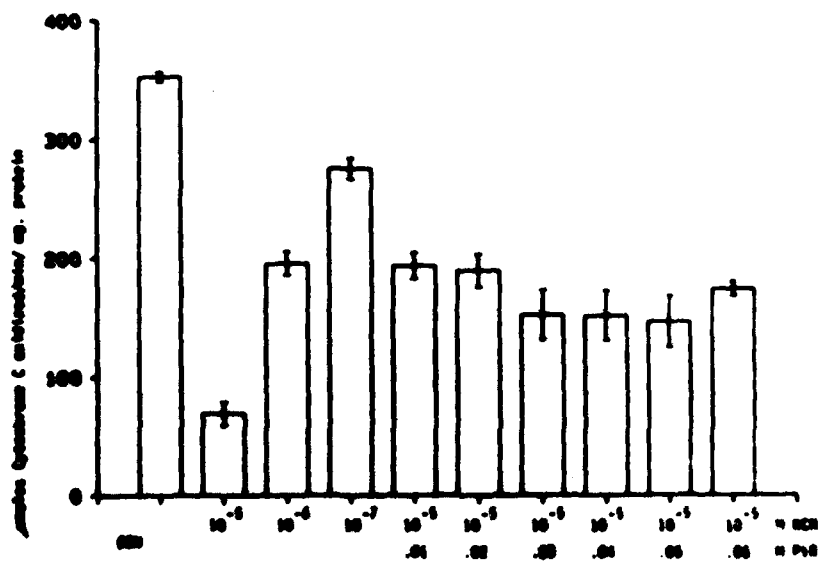


FIGURE 15.

Prevention of cyanide (CN) inhibition of cytochrome oxidase by pyruvic acid (PVR).

In Figure 15 it is noted that PYR was not particularly effective in preventing the cyanide inhibition of cytochrome oxidase. A concentration of PYR of 0.06 M was only 30% effective.

This ability of α -keto acids could be due to binding CN and preventing access of CN to the mitochondrial site of the cytochrome oxidase system. β -KG was ineffective in preventing the inhibition of cytochrome oxidase by CN. This is an interesting finding considering the fact that the chemical structure of β -KG and α -KG are so similar (differing only in the position of the carbonyl group).

This effectiveness in prevention of cyanide inhibition of cytochrome oxidase by α -KG and α -KB correlates with the binding abilities and prevention of the lethal effects of CN.

VI. Effect of α -Keto Acids on Rhodanese Activity

Rhodanese is a transulfurase which catalyzes the transformation of cyanide to thiocyanate (Vennesland et al., 1982). The rate and extent of this transformation is significant as a means of detoxifying cyanide. The possibility of effect on this enzyme by α -keto acids was investigated.

Groups of ICR mice (20-24 g) were used. The animals received by i.p. injection, saline, CN and pretreatment with α -KG 15 minutes prior to injection of CN. The animals were decapitated one minute after the injection of the CN.

Livers were quickly removed from control mice, CN, α -keto acid and α -keto acid/CN treated mice. Rhodanese was extracted and purified from the livers according to the method of Sorbo (1950). The results of these studies are shown in Table 4.

TABLE 4.

Effect of α -Ketoglutaric Acid on Rhodanese Activity

| Group | Treatment | SCN (μ moles) | SCN (μ g/mg/protein) |
|-------|-----------------------|--------------------|---------------------------|
| 1 | none | | 616.23 |
| 2 | cyanide | | 918.30 |
| 3 | α -KG | | 906.76 |
| 4 | cyanide/ α -KG | | 907.94 |

From table 4, there was no statistical difference between the measured amounts of thiocyanate produced in the animals treated with CN/ α -KG acid and those treated with CN and α -KG. It is of interest that there is an increase in the production of thiocyanate with α -KG. This could be an additional antidotal effect of α -KG. This aspect of this study needs to be explored further.

From these data, it does not appear to be any effect by the α -keto acid on rhodanese activity even when CN is present. Apparently, rhodanese activity is not inhibited by the α -keto acid.

VII. Effect of Low Doses (0.05 gm/kg) of α -Ketoglutaric Acid (α -KG) on Lethality Produced by Cyanide (CN)

The purpose of this group of experiments was to determine the effectiveness of α -KG at doses lower than the 2.0 g/kg dose as previously used.

Groups of 10 ICR male mice (20-24 g) were used. An LD_{50} curve for CN was established in order to determine an LD_{80} dose to be used for antidotal studies of α -KG. Calculations were according to the method of Litchfield and Wilcoxon (1948). A dose of 9.5 mg/kg of KCN, i.p., was determined to be the LD_{80} . Sodium thiosulfate, in doses of 0.1 g/kg and 0.05 g/kg was administered 15 minutes prior to the injection of potassium cyanide. The dose of 0.05 g/kg of sodium thiosulfate was determined to be adequate. Intraperitoneal doses of 0.025 g/kg and 0.05 g/kg of α -KG were used. Then groups of mice were pretreated with 0.05 g/kg of sodium thiosulfate and 0.05 g/kg of α -KG.

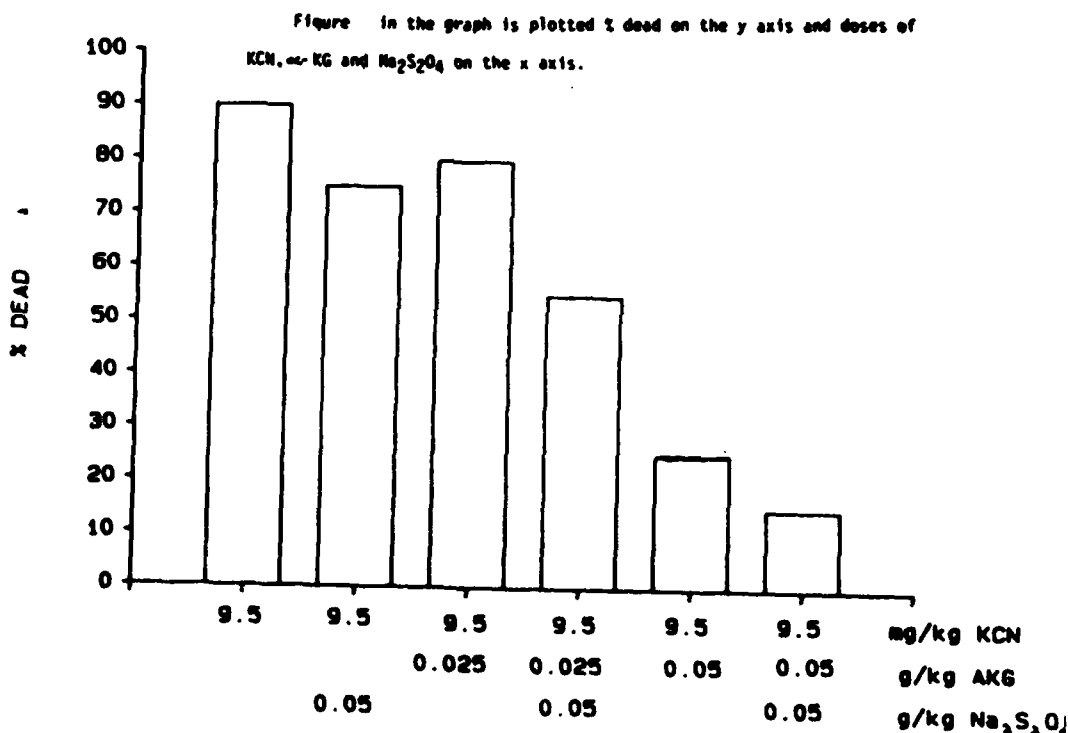


FIGURE 16.

Prevention of Lethality by Low Doses of α -Ketoglutaric Acid and/or sodium thiosulfate

From Figure 16 it can be observed that this dosage when used to antagonize the lethal effects of CN resulted in the survival of 17 of 20 mice dosed in 9.5 mg/kg (LD_{80}) of KCN. A dose of 0.05 gm/kg of α -KG alone resulted in the survival of 15/20 mice pretreated with CN.

Increasing the dose of α -KG from 0.025 to 0.05 g/kg increased survivability of the animals by 40%; adding sodium thiosulfate to α -KG increased the survivability by 60%. These findings would indicate that an assured supply of sulfone sulfur atoms is essential to antidoting CN.

Figure in the graph is plotted % dead on the y axis and doses of KCN, α -KG and $\text{Na}_2\text{S}_2\text{O}_4$ on the x axis.

VIII. The Effects of α -Ketoglutaric Acid (α -KCN) on the Production of Methemoglobin

It is recognized that methemoglobin can bind CN and, act as a cyanide antidote. This series of experiments was done to determine if an α -keto acid, α -KG, in some manner enhanced the formation of methemoglobin which would then bind the CN rather than binding CN itself. In order to standardize the procedure, nitrites, known to produce methemoglobin, were studied.

Five groups of five male ICR mice (22-24 g) were injected i.p. with amyl nitrite (20 mg/kg), sodium nitrite (80 mg/kg, i.p.), dimethylaminophenol (DMAP) (49 mg/kg, i.p.) α -ketoglutaric acid (2.0 gm/kg, i.p.). Blood was collected from a group of mice at each time point. Methemoglobin determinations were made according to the method of Evelyn and Malloy (1938) as described by Fairbanks and Klee (1986). In Figures 17a, 17b and 17c are illustrated the results of these studies. It can be seen that amyl nitrite produces methemoglobin very rapidly (over 40% methemoglobin in 5 min) with a short duration of action. The administration of sodium nitrite resulted in a level above 20% methemoglobin for at least one hour. Dimethylaminophenol maintained a level above 20% for 15 min at this dose level. No methemoglobin production was seen in the samples of blood collected from the group treated with α -ketoglutaric acid, so no graph is presented.

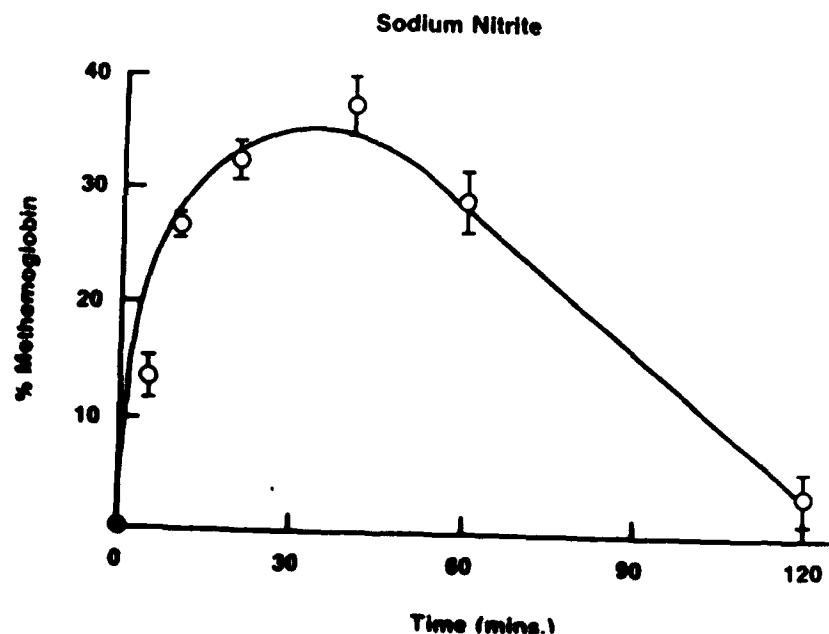


FIGURE 17a.

Production of Methemoglobin

Time course of methemoglobin production by sodium nitrite (40 mg/kg).

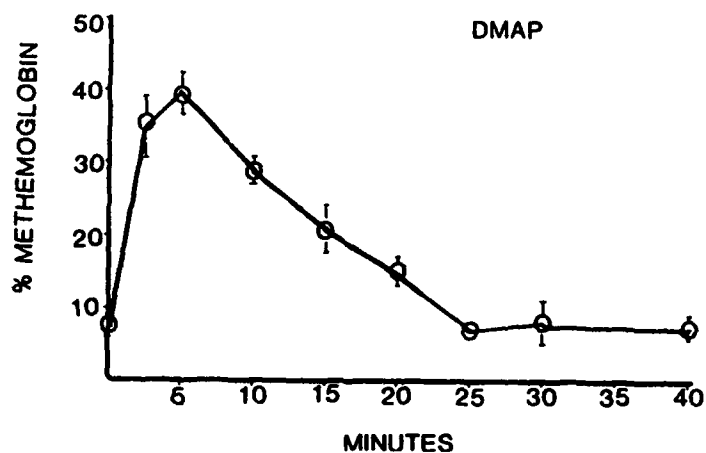


FIGURE 17b.

Production of Methemoglobin

Time course of methemoglobin production by dimethylaminophenol (DMAP (20 mg/kg)).

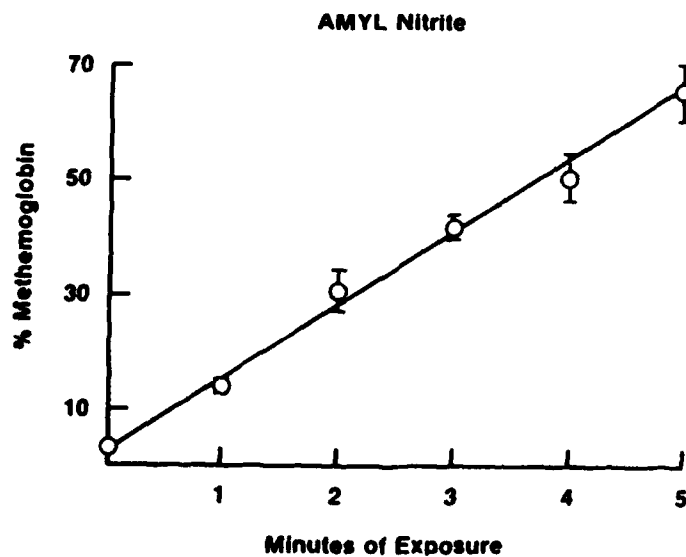


FIGURE 17c.

Production of Methemoglobin

Time course of methemoglobin production by amyl nitrite.

IX. The Effects of α -Keto Acids on the Production of Thiocyanate

Two groups of ICR mice (20-24 g) were selected. Each group contained 20 mice each. One group received CN (5.0 mg/kg) only and the second group received CN and α -KG (1.0 gm/kg). Plasma samples were obtained by decapitation of the surviving animals at specified time points. Five pooled blood samples were centrifuged to obtain plasma.

Plasma thiocyanate levels were determined on the plasma samples by the method of Hernvich and Saunders (1948) as modified by Isom et al. (1982).

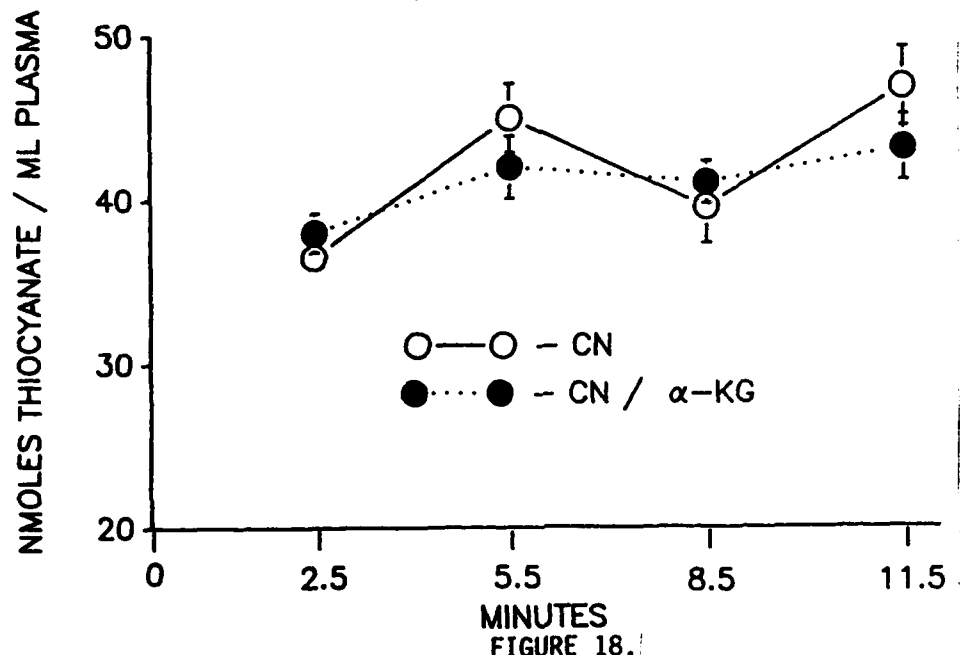


FIGURE 18.
The effect of α -ketoglutaric acid upon the metabolism of cyanide.

It is observed that there was no significant change in the production of thiocyanate between the CN treated and CN/ α -KG treated animals.

X. Effect of α -Ketoglutaric Acid (α -KG) on Cyanide (CN)-Produced-Metabolic Acidosis

A decrease in pH of blood is characteristic of CN toxicity (Graham et al., 1977). This acidosis is the result of the hypoxia and increased production of lactic acid. The extent of this acidosis is dose dependent and correlative with the severity of cyanide intoxication.

Groups of five male ICR mice were dosed with 2.0, 3.0, 4.0 and 5.0 mg/kg KCN, i.p. The animals were decapitated 2.5, 5, 5, 8, 5 and 11.5 min after CN challenge to collect blood samples for determination of pH, PCO_2 and bicarbonate contents.

Measurements were made on an Instrumentation Laboratories Blood Gas Analyzer Model 1306/282 Co-Oximeter. Calibration of the instruments were made prior to analyses of samples according to the suppliers' directions.

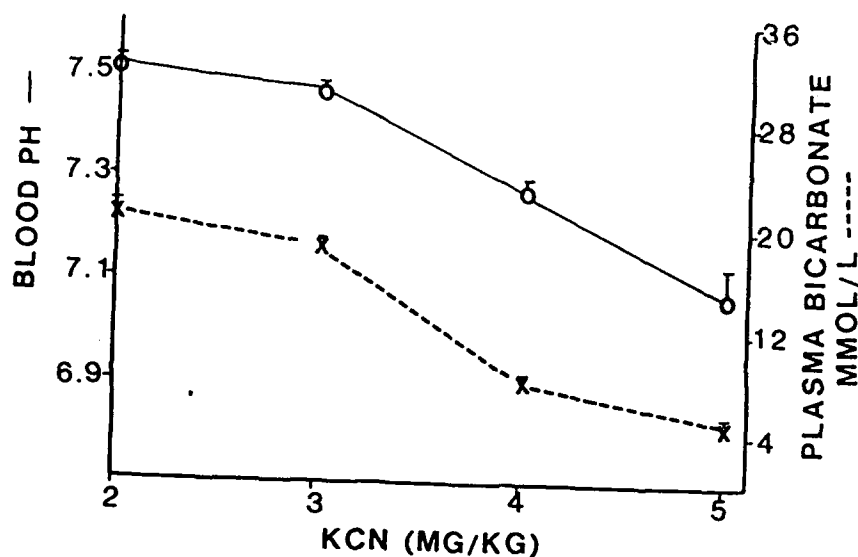


FIGURE 19.

The effect of cyanide intoxication upon blood pH and plasma bicarbonate concentration.

The data in Figure 19 show that the administration of KCN produces a dramatic decrease in blood pH and plasma bicarbonate at a dose of 4 mg/kg. Increasing the dose to 6 mg/kg KCN produces a pH inconsistent with life.

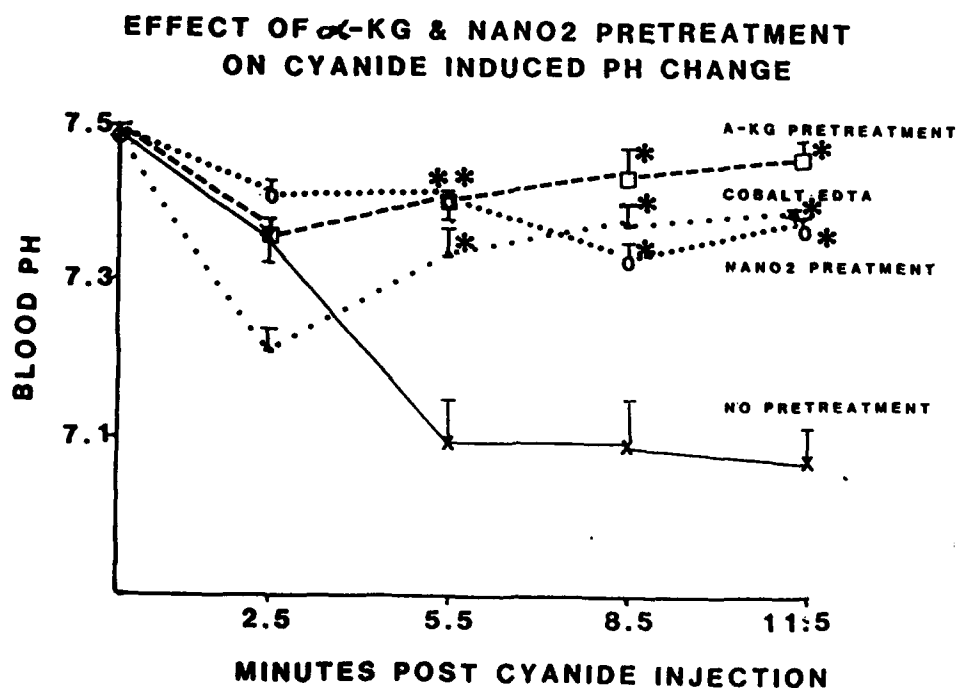


FIGURE 20.

The effect of α -ketoglutaric acid, sodium nitrite, and cobalt EDTA pretreatment upon the acidosis produced by cyanide intoxication.

Figure 20, presents data of blood pH after a dose of KCN (5 mg/kg) after pretreatment with α -KG (1.0 gm/kg), sodium (NaNO_2), 40 mg/kg, or cobalt EDTA (20 mg/kg). All three CN antagonists prevented the tremendous decrease in blood pH as is observed with cyanide alone. Cobalt EDTA is recognized to bind CN. Sodium nitrite forms methemoglobin which binds cyanide. α -KG also is proposed to bind CN, thus preventing histotoxic hypoxia and resulting acidosis.

XI. Effects of α -Ketoglutaric acid (α -KG) in Antagonizing Toxic Effects of Cyanide (CN) in Dogs

Three male beagle laboratory-raised dogs (11/13 kg) were used in these experiments. The dogs were anesthetized with thiamylal for the surgical procedure. The procedures consisted of annulation of (1) both left and right femoral veins, for i.v. dosing of KCN and antidotes and venous blood samples; (2) both left and right femoral arteries for monitoring arterial blood pressure and sampling for blood gas measurements; (3) jugular vein for cardiac catheter implantation. Cardiac output was determined by thermodilution. Heart rate, contractility, mean arterial pressure, left ventricular pressure and mean pulmonary arterial pressure were recorded using a DRA Electronics for Medicine monitor. Leads for recording for electrocardiograph were placed for lead IV.

Blood samples were obtained for quantitation of cyanide and measurement of blood pH, bicarbonate, carbon dioxide, hemoglobin, oxyhemoglobin, volume percentage and partial pressure of oxygen.

Potassium cyanide was infused first in two animals at a rate of 0.2 mg/kg/min until the toxic effects of CN on the heart were observed, i.e., tachycardia, arrhythmias, etc.

α -KG was injected as a bolus (1.0 mg/kg over 2 min) either 2 min prior or post infusion of CN. Once the cardiovascular parameters, i.e., arrhythmias, had diminished or reversed, the infusion of KCN was restarted and the procedure repeated until the effects in animal became irreversible. At this point, the animal was euthanized with thiamylal/succinylcholine.

Blood gas measurements were made according to the manufacturer's procedures using an IL 1306 Blood Gas Analyzer interfaced with an IL 282 Co-oximeter (Instrumentation Laboratories, Cambridge, MA). These instruments were calibrated prior to use according to the manufacturer's directions.

Cyanide contents of blood were determined by the method of Darr, Capson and Hileman (1980). This method utilizes a Hewlett Packard 5880 gas chromatograph equipped with nitrogen phosphorous detector. One tenth of a milliliter of blood is treated with phosphoric acid to free the cyanide from the blood in an air-tight reaction vial. Five hundred microliters of vial headspace air is injected into the gas chromatograph. The response obtained is compared with a standard concentration curve for calculation of quantity of cyanide present.

TABLE 5

Effects of Cyanide and -KG Treatment on Blood Gas Values in Dog

| Sample | CN Level (ug/ml) | HCO ₃ | TCO ₂ | Beb | SBC | BE ecf | Hb | %O ₂ HB ² | Vol %O ₂ | pH | PCO ₂ | PO ₂ | %S O ₂ M |
|--------|---------------------|------------------|------------------|-------|------|-----------|------|------------------------------------|------------------------|-------|------------------|-----------------|------------------------|
| 0 | No Peaks | 19.5 | 20.4 | -3.0 | 22.5 | -5.2 | 16.1 | 96.9 | 21. | 7.421 | 29.6 | 167 | 98.9 |
| 1 | .724 ug/ml | 12.2 | 13.0 | -11.7 | 15.8 | -143 | 13.0 | 96.8 | 17. | 7.303 | 24.4 | 177 | 99.2 |
| 2 | .593 ug/ml | 14.8 | 15.6 | -8.4 | 18.3 | -10.9 | 14.1 | 96.5 | 19.1 | 7.353 | 26.3 | 171 | 99.0 |
| 3 | .876 ug/ml | 14.8 | 15.5 | -7.4 | 19.1 | -10.2 | 13.8 | 96.9 | 18 | 7.402 | 23.5 | 175 | 99.0 |
| 4 | 3.048 ug/ml | 10.0 | 10.5 | -10.4 | 16.8 | -14.7 | 16.3 | 96.6 | 22. | 7.418 | 15.4 | 184 | 99.2 |
| 5 | 5.1 ug/ml | 6.5 | 6.9 | -14.4 | 13.7 | -18.9 | 15.2 | 96.4 | 20. | 7.373 | 11.1 | 191 | 99.0 |
| 6 | 2.317 ug/ml | 7.6 | 8.6 | -22.7 | 7.0 | -24.3 | 14.4 | 90.5 | 18. | 6.973 | 32.4 | 109 | 92.04 |
| 7 | 1.94 ug/ml | 6.1 | 7.1 | -25.6 | 4.8 | -27.0 | 14.2 | 91.6 | 18. | 6.776 | 34.3 | 96 | 83.9 |
| 8 | 1.68 ug/ml | 5.1 | 6.1 | -28.9 | 2.0 | 30.0 | 13.1 | 82.4 | 15. | 6.896 | 31.2 | 119 | 93.1 |

HB = Total Hemoglobin

%O₂HB = OxygenVol %O₂ = Oxygen

pH = pH

pCO₂ = partial pressure Carbon DioxidePO₂ = partial pressure oxygenHCO₃ = Actual Bicarbonate

BEeb = Base excess

BE ecf = Base excess in extra-cellular fluid

SBC = Std. Bicarbonate

%SO₂m = Oxygen saturatedTCO₂ = Total CO₂

Effects on Blood Gas Parameters: From the values in Table 5, it is significant that the percentages of oxyhemoglobin, carboxyhemoglobin and methemoglobin remained constant (96-97%) throughout the experiment. As expected with cyanide, the volume of oxygen increased from 11.6% to 27.1% while the partial pressure of oxygen decreased only slightly. The partial pressure of carbon dioxide decreased from 38.9 to 15.4. The pH of the blood remained fairly consistent until the blood level reached 3.0-3.5 mg (see table).

Effects on Cardiovascular Toxic Effects: This portion of the work and report were prepared with the guidance of Angel Markov, M.D., Department of Medicine, University Medical Center.

In the dogs in which α -KG was administered prior to the infusion of cyanide, it appears that the α -KG protects or prevents the occurrence of deleterious effects of CN on conduction properties of the heart. This finding is evidenced by the EKG recordings in which no effect of A-V dissociation occurs, indicating that the bundle of his is intact and functional.

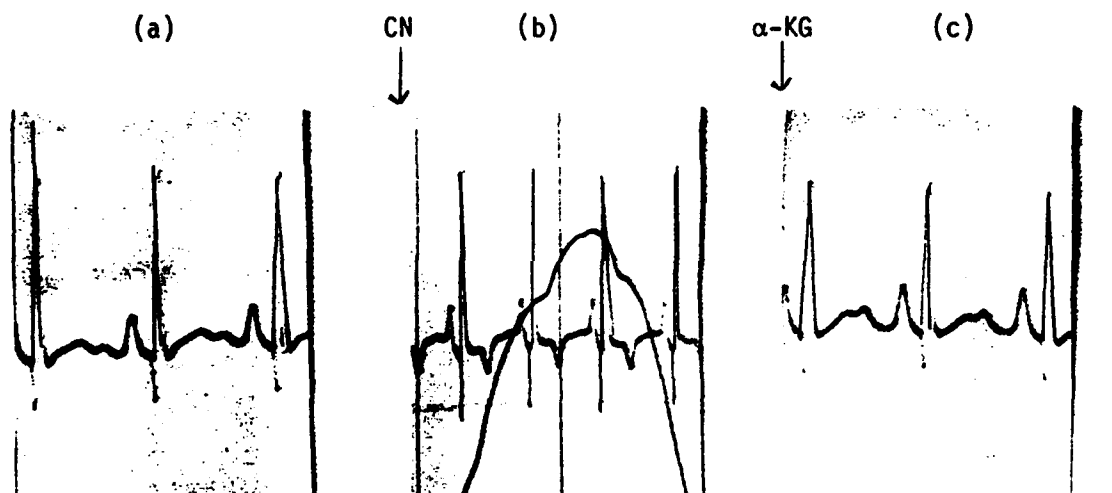


FIGURE 21.

Electrocardiographic recording during experiment with laboratory raised beagle dog. (a) control, (b) after infusion i.v. of KCN for 4 minutes (0.2 mg/kg/min), (c) after bolus injection, i.v. of α -KG (0.5 gm/kg).

The animal remained hemodynamically stable even at a 1.0 mg/kg dose (considered as a lethal dose by this method). There was an increase in cardiac output as expected. The mean arterial pressure was satisfactory but declined with very high doses of CN. At a very late stage of CN infusion (CN blood level of 4-5 mcg/ml), there was noted a significant increase in left ventricular and diastolic pressure. There was a more pronounced effect of cyanide as the dosage of CN increased to 4 mg/kg, pulmonary arterial pressure was abnormally high, however, the systemic hemodynamics were almost normal. There was an increase in pulmonary arterial pressure.

Pretreatment with α -KG appears to prevent circulatory collapse and protects the myocardium from the effects of CN.

The fact that the α -KG-treated animal was able to survive four times the lethal dose before noticeable cardiovascular effects indicates that α -KG is effective as a prophylactic agent in cyanide poisoning.

In the animal in which α -KG was administered after the infusion of CN was started, the effects of CN on the hemodynamics occurred much earlier and were more pronounced. At that point, α -KG alters the effects of CN on systemic and pulmonary hemodynamics, for the system is restabilized by α -KG infusion.

It should be emphasized that even post-CN treatment with α -KG is effective in counteracting the toxic effects of CN. This is evidenced by the fact that the dog received 4 times the lethal dose of CN before irreversible cardiovascular effects appeared.

XII. Effects of Cyanide on 2,3-Diphosphoglyceric Acid

It is proposed that hemoglobin-oxygen association is affected by the enzymically catalyzed production of 2,3-diphosphoglyceric acid. These experiments were designed to test the possibility of inhibition of the enzyme phosphoglycerate mutase by cyanide or by α -keto acids themselves. Groups of mice (5 each) were selected as control, cyanide treated, α -keto acid treated and cyanide/ α -keto acid treated. Concentration of 2,3-diphosphoglyceric acid was determined according to the procedure in Procedure #665, Sigma Diagnostics. In this procedure one milliliter of heparinized animal blood was required for each test.

The sample was treated with enzyme, triethanolamine buffer and phosphoglycolic acid solution. Finally, Fiske and Suborow solution is added for color development. The intensity of color is measured spectrophotometrically and absorbance is compared to a standard concentration curve.

No significant difference in control and CN-treated samples in concentrations of 2,3 diphosphoglyceric acid was detected.

TABLE 6.

| Sample | Concentration of 2,3 diphosphoglyceric acid (μ M/ml) |
|-----------------|---|
| Control | 1.84 \pm 0.3 |
| Cyanide treated | 1.76 \pm 0.2 |

XIII. Stability of Aqueous Solutions α -KG

Since α -KG was prepared in aqueous solution for injection, the stability of the solutions of α -KG was determined. A solution of α -KG prepared thirty days prior to use was compared for cyanide antagonism efficacy with freshly prepared solution of α -KG of the same concentration. One group of mice were injected with doses (2 g/kg) from the freshly prepared solution of α -KG. A second group were injected with doses (2 g/kg) from the month-old solution of α -KG.

TABLE 7.
Stability of Solution of α -Ketoglutaric Acid

| Dose of KCN, mg/kg | Lethality Results | |
|--------------------|---------------------------------------|--|
| | Month old solution of α -KG | Freshly prepared solution of α -KG |
| 23.0 | 7/10* | 7/10* |
| 25.0 | 5/10 | 4/10 |

* Results are expressed as survivors over total number of animals dose. From this experiment, it can be concluded that there is no significant deterioration of α -KG in aqueous solution over a 30 day period of time.

From the lethality studies in Table 7 the solutions of α -KG prepared thirty days previous to their use were equal in effectiveness as the freshly prepared solution.

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XV. Personnel

1. Dr. James C. Norris, Post Doctoral
2. Mr. Steven J. Moore, Graduate student awarded Doctor of Philosophy degree, August 1986.
3. Ms. Robin Hubbuch, Research associate

XVI. Publications - wholly or partially supported by this contract

1. Moore, S.J., J.C. Norris, I.K. Ho, and A.S. Hume, 1986. The efficacy of α -ketoglutaric acid in the antagonism of cyanide intoxication. Toxicol. and Appl. Pharmacol. 82,(1):40.
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